complementary to a second portion of said preselected nucleic acid target sequence, and

said core region having a sequence according to the formula:

(I.) T(stem)'AGC(stem)"Z,

E27

wherein said (stem)' and (stem)" are each three sequential nucleotides which when hybridized as a (stem)':(stem)" pair comprise three base pairs including at least two G:C pairs and wherein said Z = WCGR or WCGAA, and W = A or T and R = A or G; or

(II.) RGGCTAGCHACAACGA (SEQ ID NO 122),

wherein said H = T, C or A, and R = A or G.

At page 145, line 31, please substitute the following claim 35 for the previously submitted claim 35:

E28

35. The method of claim 34 wherein said exonuclease-resistant nucleotides comprise nucleoside phosphorothioate.

REMARKS

The amendments detailed above provide unique sequence identifiers for each sequence disclosed in the specification and corresponding sequence listing as required under 37 C.F.R. §§ 1.1821 - 1.825. The amendments are supported by the sequence disclosures in the specification. No new matter is added by way of the present response. Applicants respectfully request entry of the amendments and sequence listing provided herein and in supporting documents, including the CRF.

Applicants believe that the present response is a bona fide attempt to advance the application, and is a complete reply to the Notice to Comply with Requirements For Patent Applications Containing Nucleotide sequence and/or Amino Acid Sequence Disclosures mailed October 22, 2002. Therefore, if consideration of some matter or compliance with some requirement has been inadvertently omitted, Applicants respectfully request that the Examiner provide a new time period for reply under 37 C.F.R. § 1.134 to supply the omission.

The Examiner is requested to contact the representative for the Applicants, to discuss any questions or for clarification.

If there are any further fees associated with this response, the Director is authorized to charge our Deposit Account No. 19-0962.

January 21, 2003
Date

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APPENDIX

VERSION WITH MARKINGS SHOWING CHANGES MADE

In The Specification:

At Page 15, line 27, please amend the paragraph as follows:

Figure 1 illustrates a selective amplification scheme for isolation of DNAs that cleave a target RNA phosphoester. As shown, double-stranded DNA that contains a stretch of 50 random nucleotides (the molecule with "N_{50}" (SEQ ID NO 145) indicated above it) is amplified by PCR, employing a 5′-biotinylated DNA primer that is terminated at the 3′ end by an adenosine ribonucleotide (rA). (The biotin label is indicated via the encircled letter "B".) This primer is extended by *Taq* polymerase to yield a DNA product that contains a single embedded ribonucleotide. The resulting double-stranded DNA is immobilized on a streptavidin matrix and the unbiotinylated DNA strand is removed by washing with 0.2 N NaOH. After re-equilibrating the column with a buffered solution, the column is washed with the same solution with added 1 mM PbOAc. DNAs that undergo Pb^{2+}-dependent self-cleavage are released from the column, collected in the eluant, and amplified by PCR. The PCR products are then used to initiate the next round of selective amplification.

At Page 16, line 20, please amend the paragraph as follows:

Figure 3 illustrates the sequence alignment of individual variants isolated from the population after five rounds of selection [(SEQ ID NOs 13-22)]. The fixed substrate domain is shown at the top, with the target riboadenylate identified via an

inverted triangle (SEQ ID NO 13). Substrate nucleotides that are commonly involved in presumed base-pairing interactions are indicated by vertical bars. Sequences corresponding to the 50 initially-randomized nucleotides are aligned antiparallel to the substrate domain. All of the variants are 3′-terminated by the fixed sequence 5′-CGGTAAGCTTGGCAC-3′ (not shown; SEQ ID NO 1). Nucleotides within the initially-randomized region that are presumed to form base pairs with the substrate domain are indicated on the right and left sides of the Figure; the putative base-pair-forming regions of the enzymatic DNA molecules are individually boxed in each sequence shown. Conserved regions are illustrated via the two large, centrally-located boxes.

At Page 17, line 4, please amend the paragraph as follows:

Figures 4A and 4B illustrate DNA-catalyzed cleavage of an RNA phosphoester in an intermolecular reaction that proceeds with catalytic turnover. Figure 4A is a diagrammatic representation of the complex formed between the 19mer substrate (5[3]'-TCACTATrAGGAAGAGATGG-3[5]', SEQ ID NO 2) and 38mer DNA enzyme (5'-ACACATCTCTGAAGTAGCGCCGCCGTATAGTGACGCTA-3', SEQ ID NO 3). The substrate contains a single adenosine ribonucleotide ("rA", adjacent to the arrow), flanked by deoxyribonucleotides. The synthetic DNA enzyme is a 38-nucleotide portion of the most frequently occurring variant shown in Figure 3. Highly-conserved nucleotides located within the putative catalytic domain are "boxed". As illustrated, one conserved sequence is "AGCG", while another is "CG" (reading in the 5'-3' direction).

At Page 18, line 9, please amend the paragraph as follows:

Figures 6A and 6B provide two-dimensional illustrations of a "progenitor" catalytic DNA molecule and one of several catalytic DNA molecules obtained via the selective amplification methods disclosed herein, respectively. Figure 6A illustrates an exemplary molecule from the starting pool, showing the overall configuration of the molecules represented by SEQ ID NO 133 [from residue nos. 21-92]. As illustrated, various complementary nucleotides flank the random (N_{40}) (SEQ ID NO 143) region. Figure 6B [(SEQ ID NO 123)] is a diagrammatic representation of one of the Mg^{2+}-dependent catalytic DNA molecules (or "DNAzymes") (SEQ ID NO 123) generated via the within-described procedures. The location of the ribonucleotide in the substrate nucleic acid is indicated via the arrow in both Figs. 6A and 6B.

At Page 19, line 4, please amend the paragraph as follows:

Figure 8 illustrates the nucleotide sequences, cleavage sites, and turnover rates of two catalytic DNA molecules of the present invention, clones 8-17 (SEQ ID NO 134) and 10-23 (SEQ ID NO 136). Reaction conditions were as shown, namely, 10mM Mg^{2+}, pH 7.5, and 37°C. The DNAzyme identified as clone 8-17 [residue nos. 1-34 of SEQ ID NO 56] is illustrated on the left, with the site of cleavage of the RNA substrate indicated by the arrow. The substrate sequence (5' - GGAAAAAGUAACUAGAGAUGGAAG - 3' [residue nos. 1-24 of SEQ ID NO 51] (SEQ ID NO 135)) -- which is separate from the DNAzyme (i.e., intermolecular cleavage is shown) -- is labeled as such. Similarly, the DNAzyme identified herein as 10-23 [residue nos. 3-33 of SEQ ID NO 85] is shown on

the right, with the site of cleavage of the RNA substrate indicated by the arrow. Again, the substrate sequence is indicated (SEQ ID NO 135). For the 8-17 [residue nos. 4-30 of SEQ ID NO 56] enzyme, the turnover rate was approximately 0.6 hr^{-1}; for the 10-23 enzyme, the turnover rate was approximately 1 hr^{-1}. Noncomplementary pairings are indicated with a closed circle (●), whereas complementary pairings are indicated with a vertical line (|).

At Page 19, line 22, please amend the paragraph as follows:

Figure 9 further illustrates the nucleotide sequences, cleavage sites, and turnover rates of two catalytic DNA molecules of the present invention, clones 8-17 (SEQ ID NO 138) and 10-23 (SEQ ID NO 137). Reaction conditions were as shown, namely, 10mM Mg^{2+}, pH 7.5, and 37°C. As in Figure 8, the DNAzyme identified as clone 8-17 [residue nos. 4-30 of SEQ ID NO 56] is illustrated on the left, with the site of cleavage of the RNA substrate indicated by the arrow. The substrate sequence (5' - GGAAAAAGUAACUAGAGAUGGAAG - 3' [residue nos. 1-24 of SEQ ID NO 51] (SEQ ID NO 135)) --which is separate from the DNAzyme (i.e., intermolecular cleavage is shown) -- is labeled as such. Similarly, the DNAzyme identified herein as 10-23 residue nos. 5-33 of SEQ ID NO 85, with "CTA" substituted for "TTG" at the 5' end is shown on the right, with the site of cleavage of the RNA substrate indicated by the arrow. Again, the substrate sequence is indicated (SEQ ID NO 135). For the 8-17 enzyme, k_{obs} was approximately 0.002 min^{-1}; for the 10-23 enzyme, the value of k_{obs} was approximately 0.01 min^{-1}. Noncomplementary pairings are indicated with a closed circle (●), whereas complementary

pairings are indicated with a vertical line (|).

At Page 20, line 7, please amend the paragraph as follows:

Figure 10 illustrates a schematic showing the composition of the 8-17 [residue nos. 10-23 of SEQ ID NO 56] (SEQ ID NO 120) and 10-23 [SEQ ID NO 122] (SEQ ID NO 121) catalytic motifs. The DNA enzyme (bottom strand) binds the RNA substrate (top strand) through complementary Watson-Crick pairing (vertical lines) between unspecified complementary nucleotides (horizontal lines). Cleavage occurs at the position indicated by the arrow, where R = A or G and Y = U or C.

At Page 59, line 26, please amend the paragraph as follows:

Figure 3 illustrates the sequence alignment of individual variants isolated from the population after five rounds of selection. The fixed substrate domain (5'-GGGACGAATTCTAATACGACTCACTATrAGGAAGAGATGGCGAC-3' (SEQ ID NO:13), or 5'-GGGACGAATTCTAATACGACTCACTATNGGAAGAGATGGCGAC-3', where N represents adenosine ribonucleotide) (SEQ ID NO 13) is shown at the top, with the target riboadenylate identified with an inverted triangle. Substrate nucleotides that are commonly involved in presumed base-pairing interactions are indicated by a vertical bar. Sequences corresponding to the 50 initially-randomized nucleotides are aligned antiparallel to the substrate domain. All of the variants are 3'-terminated by the fixed sequence 5'-CGGTAAGCTTGGCAC-3' (SEQ ID NO 1) ("primer site"; not shown). Nucleotides within the initially-randomized region that are presumed to form base pairs with the substrate

domain are indicated on the right and left sides of the Figure; the putative base-pair-forming (or substrate binding) regions of the enzymatic DNA molecules are individually boxed in each sequence shown. The highly-conserved nucleotides within the putative catalytic domain are illustrated in the two boxed columns.

At Page 61, line 28, please amend the paragraph as follows:

Synthetic DNAs and DNA analogs were purchased from Operon Technologies. The 19-nucleotide substrate, 5'-pTCACTATrAGGAAGAGATGG-3' (SEQ ID NO:7) (or 5'-pTCACTATNGGAAGAGATGG-3', wherein "N" represents adenosine ribonucleotide) (SEQ ID NO 7), was prepared by reverse-transcriptase catalyzed extension of 5'-pTCACTATrA-3' (SEQ ID NO:8) (or 5'-pTCACTATN-3', wherein "N" represents adenosine ribonucleotide) (SEQ ID NO 8), as previously described (Breaker et al, Biochemistry, 33:11980-11986, 1994), using the template 5'-CCATCTCTTCCTATAGTGAGTCCGGCTGCA-3' (SEQ ID NO 9). Primer 3, 5'-GGGACGAATTCTAATACGACTCACTATrA-3' (SEQ ID NO:6) (or 5'-GGGACGAATTCTAATACGACTCACTATN-3', wherein "N" represents adenosine ribonucleotide) (SEQ ID NO 6), was either 5'-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (primer 3a) or 5'-thiophosphorylated with [γ-S]ATP and T4 polynucleotide kinase and subsequently biotinylated with *N*-iodoacetyl-*N'*-biotinylhexylenediamine (primer 3b).

At Page 66, line 2, please amend the paragraph as follows:

In designing the catalytic domain, we relied heavily on the

composition of the most reactive variant, truncating by two nucleotides at the 5' end and 11 nucleotides at the 3' end. The 15 nucleotides that lay between the two template regions were left unchanged and a single nucleotide was inserted into the 3' template region to form a continuous stretch of nucleotides capable of forming base pairs with the substrate. The substrate was simplified to the sequence 5'-TCACTATrA • GGAAGAGATGG-3' (SEQ ID NO:12) (or 5'-TCACTATN • GGAAGAGATGG-3', wherein "N" represents adenosine ribonucleotide) (SEQ ID NO 12), where the underlined nucleotides correspond to the two regions involved in base pairing with the catalytic DNA molecule.

At Page 71, line 24, please amend the paragraph as follows:

Individual clones were isolated following the sixth round, and the nucleotide sequence of 24 of these clones was determined. All of the sequences began with: 5′ GGG ACG AAT TCT AAT ACG ACT CAC TAT rA GG AAG AGA TGG CGA CA (SEQ ID NO 139 [23 from position 1 to 44]) and ended with: CGG TAA GCT TGG CAC 3′ (SEQ ID NO 1 [23 from position 93 to 107]).

At Page 71, line 30, please amend the paragraph as follows:

The segment in the middle, corresponding to TCTC $\mathbf{N}_{40}$ GTGA (SEQ ID NO 140 [23 from position 45 to 92]) in the starting pool, varied as follows:

At Page 73, line 16, please amend the paragraph as follows:

The lead compound was re-randomized at 40 nucleotide

positions (underlined), introducing mutations at a frequency of 15% (5% probability of each of the three possible base substitutions). The re-randomized population was subjected to seven additional rounds of *in vitro* selection. During the last four rounds, molecules that were reactive in the presence of 1 mM Pb^{2+} were removed from the population before the remainder were challenged to react in the presence of 1 mM Mg^{2+}. Individual clones were isolated following the seventh round and the nucleotide sequence of 14 of these clones was determined. All of the sequences began with: 5′ GGG ACG AAT TCT AAT ACG ACT CAC TAT **rA** GG AAG AGA TGG CGA CAT CTC (SEQ ID NO 141 [23, from position 1 to 48]), and ended with: GTG ACG GTA AGC TTG GCA C 3′ (SEQ ID NO 142 [23, from position 89 to 107]).

At Page 73, line 31, please amend the paragraph as follows:

The segment in the middle, corresponding to the 40 partially-randomized positions (N_{40}, SEQ ID NO 143 [23, from position 49 to 88]), varied as follows:

At Page 74, line 28, please amend the paragraph as follows:

Figures 6A and 6B provide two-dimensional illustrations of a "progenitor" catalytic DNA molecule and one of several catalytic DNA molecules obtained via the selective amplification methods disclosed herein, respectively. Figure 6A illustrates an exemplary molecule from the starting pool, showing the overall configuration of the molecules represented by SEQ ID NO 23. As illustrated, various complementary nucleotides flank the random (N_{40}) (SEQ ID NO 143) region.

At Page 76, line 11, please amend the paragraph as follows:

The initial library was generated by template-directed extension of 50 pmols of 5'-biotin-d(GGAAAAA)r(GUAACUAGAGAU)d(GGAAGAGATGGCGAC)-3' [(residue nos. 1-34 of SEQ ID NO 51)] (SEQ ID NO 144) on 100 pmols of 5'-GTGCCAAGCTTACCG-N50-GTCGCCATCTCTTCC-3' (SEQ ID NO 4) (N = G, A, T or C), in a 50-ul reaction mixture containing 10 U ul^{-1} Superscript II reverse transcriptase (RT; Gibco BRL), 3 mM MgCl2, 75 mM KCl, 50 mM Tris*HCl (pH 8.3), and 0.2 mM of each dNTP. A trace amount of [5'-32P]-labeled primer was included in the reaction mixture to allow extension efficiency to be monitored. All components except RT were combined, incubated at 65 ▒C for 5 min, then cooled to 45 ▒C over 10 min. RT was added and the mixture was incubated at 45 ▒C for 45 min, then quenched by addition of Na2 EDTA. NaCl was added to a final concentration of 1 M and the extension products were immobilized by repeated passing through four streptavidin affinity columns (Genosys). The columns were washed with five 100-ul volumes of wash buffer (1 M NaCl, 50 mM Tris*HCl (pH 7.5), 0.1 mM Na2EDTA), followed by five 100-ul volumes of 0.1 N NaOH and five 100-ul volumes of wash buffer at 37 ▒C, then eluted at 37 ▒C over 1 hr with three 20-ul aliquots of reaction buffer (10 mM MgCl2, 1 M NaCl, 50 mM Tris*HCl (pH 7.5)). Eluted molecules were recovered and amplified by the polymerase chain reaction (PCR) using the primers 5'-biotin-GGAAGAGATGGCGAC-3' [(residue nos. 19-33 of SEQ ID NO 50)] (SEQ ID NO 145) and 5'-GTGCCAAGCTTACCG-3' [residue nos. 1-15 of SEQ ID NO 4)](SEQ ID NO 10). The PCR products were immobilized on streptavidin columns, as above, which were washed with five 100-ul volumes of wash buffer and eluted with 40 ul of 0.1 N NaOH

to obtain the non-biotinylated strand. The isolated DNAs were ethanol precipitated and used as templates in a primer extension reaction to begin the next round of selection. Rounds 2-10 were carried out as above, except that the reaction scale was reduced five-fold during the extension step and two-fold during PCR.

At Page 78, line 12, please amend the paragraph as follows:

Under the heading "Nucleotide Sequence" in each of Tables 2 and 3 is shown the portion of each identified clone that corresponds to the 50 nucleotides that were randomized in the starting pool (i.e., N_{50} (SEQ ID NO 146); thus, the entire nucleotide sequence of a given clone generally includes the nucleotide sequences preceding, following, and including the "N_{50}" (SEQ ID NO 146) segment, presuming the substrate sequence is attached and that self-cleavage has not occurred. For example, the entire sequence of a (non-self-cleaved) clone may generally comprise residue nos. 1-33 of SEQ ID NO 50, followed by the residues representing the randomized N_{50} (SEQ ID NO 146) region, followed by residue nos. 84-98 of SEQ ID NO 50, or by residue nos. 1-34 of SEQ ID NO 51, followed by the residues representing the randomized N_{50} (SEQ ID NO 146) region, followed by residue nos. 85-99 of SEQ ID NO 51. It is believed, however, that the N_{50} (SEQ ID NO 146) (or N_{40} (SEQ ID NO 143)) region -- or a portion thereof -- of each clone is particularly important in determining the specificity and/or activity of a particular enzymatic DNA molecule. This is particularly evident in reactions in which the substrate and the DNAzyme are separate molecules (see, e.g., Figs. 8 and 9).

At Page 78, line 33, please amend the paragraph as follows:

Clone numbers are designated as 8-x or 10-x for individuals obtained after the 8th or 10th rounds, respectively. SEQ ID NOS are also listed and correspond to the "N_{50}" (SEQ ID NO 146) region of each clone.

At Page 82, line 24, please amend the paragraph as follows:

The self-cleavage activity of various clones was subsequently measured. Clones 8-5, 8-17, and 10-3 were found to cleave efficiently at the site 5' GUAACU↓AGAGAU 3' (SEQ ID NO 49), while clones 10-14, 10-19 and 10-27 were found to cleave efficiently at the site 5' G↓UAACUAGAGAU 3' (SEQ ID NO 49). When the RNA portion of the molecule was extended to the sequence 5' GGAAAAAGUAACUAGAGAUGGAAG 3' (SEQ ID NO 135) [(residue nos. 1-24 of SEQ ID NO 51)], clones 8-17, 10-14, and 10-27 retained full activity, while clones 8-5, 10-3, and 10-19 showed diminished activity. Subsequently, clone 10-23 was found to exhibit a high level of activity in the self-cleavage reaction involving the extended RNA domain.

At Page 83, line 2, please amend the paragraph as follows:

It should also be noted, in the event one of skill in the relevant art does not appreciate same, that the nucleotide sequences preceding and following the "N_{50}" (SEQ ID NO 146) segments of the polynucleotide molecules engineered according to the teachings of the present invention disclosure, i.e, the substrate binding regions flanking the "N_{50}" (SEQ ID NO 146)

region, may be altered in a variety of ways in order to generate enzymatic DNA molecules of particular specificities, such as by length, nucleotide sequence, type of nucleic acid, and the like. For example, while residue nos. 1-24 of SEQ ID NO 51 are described herein as RNA nucleotides, they may alternatively comprise DNA, RNA, or composites thereof. (Thus, for example, SEQ ID NO 51 could easily be altered so that nucleic acid residue nos. 1-7 would comprise DNA, residue nos. 8-19 would comprise RNA, residue nos. 20-99 would comprise DNA, and so on.) Similarly, the nucleotides following the "N_{50}" (SEQ ID NO 146) region may comprise RNA, DNA, or composites thereof. The length of the regions preceding and following the "N_{50}" (SEQ ID NO 146) (or "N_{40}" (SEQ ID NO 143) -- see Example 4) region(s) may also be varied, as disclosed herein. Further, sequences preceding and/or following N_{50} (SEQ ID NO 146) or N_{40} (SEQ ID NO 143) regions may be shortened, expanded, or deleted in their entirety.

At Page 84, line 11, please amend the paragraph as follows:

Figure 8 illustrates the nucleotide sequences, cleavage sites, and turnover rates of two catalytic DNA molecules of the present invention, clones 8-17 and 10-23. Reaction conditions were as shown, namely, 10mM Mg^{2+}, pH 7.5, and 37°C. The DNAzyme identified as clone 8-17 is illustrated on the left, with the site of cleavage of the RNA substrate indicated by the arrow. The substrate sequence (5' - GGAAAAAGUAACUAGAGAUGGAAG - 3' [(residue nos. 1-24 of SEQ ID NO 51)] (SEQ ID NO 135) -- which is separate from the DNAzyme (i.e., intermolecular cleavage is shown) -- is labeled as such. Similarly, the DNAzyme identified herein as 10-23 is shown on the right, with the site of cleavage

of the RNA substrate indicated by the arrow. Again, the substrate sequence is indicated. For the 8-17 enzyme, the turnover rate was approximately 0.6 hr^{-1}; for the 10-23 enzyme, the turnover rate was approximately 1 hr^{-1}.

At Page 84, line 26, please amend the paragraph as follows:

As illustrated in Figure 8, the nucleotide sequence of the clone 8-17 catalytic DNA molecule capable of cleaving a separate substrate molecule was as follows: 5'-CTTCCACCTTCCGAGCCGGACGAAGTTACTTTTT-3' [(residue nos. 1-34 of SEQ ID NO 56)] (SEQ ID NO 134). In that same figure, the nucleotide sequence of the clone 10-23 catalytic DNA molecule capable of cleaving a separate substrate molecule was as follows: 5'-CTTTGGTTAGGCTAGCTACAACGATTTTTCC-3' [(residue nos. 3-33 of SEQ ID NO 85)] (SEQ ID NO 136).

At Page 85, line 2, please amend the paragraph as follows:

Figure 9 further illustrates the nucleotide sequences, cleavage sites, and turnover rates of two catalytic DNA molecules of the present invention, clones 8-17 and 10-23. Reaction conditions were as shown, namely, 10mM Mg^{2+}, pH 7.5, and 37°C. As in Figure 8, the DNAzyme identified as clone 8-17 is illustrated on the left, with the site of cleavage of the RNA substrate indicated by the arrow. The substrate sequence (5' - GGAAAAAGUAACUAGAGAUGGAAG - 3' [(residue nos. 1-24 of SEQ ID NO 51)] (SEQ ID NO 135)) --which is separate from the DNAzyme (i.e., intermolecular cleavage is shown) -- is labeled as such. Similarly, the DNAzyme identified herein as 10-23 is shown on the

right, with the site of cleavage of the RNA substrate indicated by the arrow. Again, the substrate sequence is indicated. For the 8-17 enzyme, k_{obs} was approximately 0.002 min^{-1}; for the 10-23 enzyme, the value of k_{obs} was approximately 0.01 min^{-1}.

At Page 85, line 17, please amend the paragraph as follows:

As illustrated in Figure 9, the nucleotide sequence of the clone 8-17 catalytic DNA molecule capable of cleaving a separate substrate molecule was as follows: 5'-CCACCTTCCGAGCCGGACGAAGTTACT-3' [(residue nos. 4-30 of SEQ ID NO 56)] (SEQ ID NO 138). In that same figure, the nucleotide sequence of the clone 10-23 catalytic DNA molecule capable of cleaving a separate substrate molecule was as follows: 5'-CTAGTTAGGCTAGCTACAACGATTTTTCC-3' (SEQ ID NO 137) (residue nos. 5-33 of SEQ ID NO 85, with "CTA" substituted for "TTG" at the 5' end).

At Page 88, line 17, please amend the paragraph as follows:

The re-selections based on the 8-17 and 10-23 molecules involved six different lineages for each motif. Each lineage entailed 5-21 rounds of in vitro selection, differing with respect to the selection protocol and reaction times. All cleavage reactions were carried out in 2 mM MgCl2, 150 mM NaCl, and 50 mM Tris*HCl (pH 7.5) at 37 C. Reaction times varied from 60 min in early rounds to 1 min in later rounds. Each starting pool of templates was based on a sequence complementary to the prototype, with fixed binding arms of seven nucleotides each and a catalytic core randomized to 25% degeneracy at each nucleotide

position. For the 8-17 and 10-23 motifs, the templates had the sequence 5'-gtgccaagcttaccgagtaactTCG-TCCGGCTCGGRagatgggtcgtctgtccttccATCT CTAGTTACTTTTTC- 3' (SEQ ID NO 124) and 5'-gttgccaagcttaccg-ggaaaaaTCGTTGTAGCTAGCCtaactaggtcgtctgtccttccA TCTCTAGT TACTTTTTC-3' (SEQ ID NO 125), respectively (PCR primer sites in lower case; substrate-binding arms underlined; randomized positions italicized). The primer used in the template-directed extensions had the sequence 5'-biotin-r(GGAAAAA-GUAACUAGAGAUGG)d(AAGAGATGGCGAC)-3' [(residue nos. 1-34 of SEQ ID NO 51)] (SEQ ID NO 132). The PCR primers for the 8-17-based selections were 5'-GTGCCAAGCTTACCGAGTAACT-3' [(residue nos. 1-22 of SEQ ID NO 124)] (SEQ ID NO 147) and 5'-d(GGAAGGACAGACGACC-CATC)rU (SEQ ID NO [126] 148)and for the 10-23-based selections were 5'-GTGCCAAGCTTACCGGGAAAAA-3' (SEQ ID NO 127) and 5'-d(GGAAGGACAGACGACCTAGTT)rA (SEQ ID NO [128] 149). The PCR primers encompassed the binding arms, thus fixing these sequences. One of the PCR primers in each set contained a 3'-terminal ribonucleotide, allowing isolation of the template strand from the double-stranded PCR products by alkaline hydrolysis of the non-template strand and subsequent purification by polyacrylamide gel electrophoresis. A gel-based selection scheme was employed in some of the lineages. In those cases, the PCR primers were 5'-biotin-GTGCCAAGCTTACCG-3' [(residue nos. 1-15 of SEQ ID NO 124)] (SEQ ID NO 150) and 5'-GAAAAAGTAACTAG-AGATGGAAGGACAGACGACC-3' (SEQ ID NO 129) and the extension reactions were carried out on the solid support using the primer 5'-r(GGAAAAAGUAACUAGAGAUGGAAG)-3' [(residue nos. 1-24 of SEQ ID NO 51)] (SEQ ID NO 135). A trace amount of [a-32P]-dATP was included in the mixture to label the extension

products, which were eluted with alkali, purified by denaturing polyacrylamide gel purification, and recovered by electroelution. The molecules then were reacted and those that underwent cleavage were isolated by gel electrophoresis.

In The Claims:

At Page 141, line 4, please amend Claim 1 as follows:

1. A catalytic DNA molecule having site-specific endonuclease activity specific for a nucleotide sequence defining a cleavage site in a preselected substrate nucleic acid sequence,

said molecule having first and second substrate binding regions flanking a core region,

wherein said first substrate binding region has a sequence complementary to a first portion of said preselected substrate nucleic acid sequence,

said second substrate binding region has a sequence complementary to a second portion of said preselected substrate nucleic acid sequence, and

said core region having a sequence according to the formula:

(I.) T(stem)'AGC(stem)"Z,

wherein said (stem)' and (stem)" are each three sequential nucleotides which when hybridized as a (stem)':(stem)" pair comprise three base pairs including at least two G:C pairs and wherein said Z = WCGR or WCGAA, and W = A or T and R = A or G; or

(II.) RGGCTAGCH[X]ACAACGA (SEQ ID NO 122),

wherein said H[X] = T, C or A, and R = A or G.

At Page 144, line 21, please amend Claim 29 as follows:

29. A method of engineering a catalytic DNA molecule that cleaves a preselected substrate nucleic acid sequence in a target nucleic acid molecule, comprising the steps of:

a) selecting a substrate nucleic acid sequence of from 10 to 26 nucleotides in length in a target nucleic acid molecule; and

b) synthesizing a deoxyribonucleic acid molecule comprising first and second substrate binding regions flanking a core region,

wherein said first substrate binding region has a sequence complementary to a first portion of said preselected nucleic acid target sequence,

said second substrate binding region has a sequence complementary to a second portion of said preselected nucleic acid target sequence, and

said core region having a sequence according to the formula:

(I.) T(stem)'AGC(stem)"Z,

wherein said (stem)' and (stem)" are each three sequential nucleotides which when hybridized as a (stem)':(stem)" pair comprise three base pairs including at least two G:C pairs and wherein said Z = WCGR or WCGAA, and W = A or T and R = A or G; or

(II.) RGGCTAGCH[X]ACAACGA (SEQ ID NO 122),

wherein said H[X] = T, C or A, and R = A or G.

At Page 145, line 31, please amend Claim 35 as follows:

35. The method of claim [7] 34 wherein said exonuclease-resistant nucleotides comprise nucleoside phosphorothioate.

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